AMENDMENTS

AMENDMENTS TO THE SPECIFICATION

In the Specification:

Please replace the second paragraph on page seven of the specification with the following substitute paragraph:

Alternatively, an alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman (Smith and Waterman, 1981, Advances in Applied Mathematics 2:482-489; database: European Bioinformatics Institute; Smith and Waterman, 1981, J. of Molec.Biol., 147:195197; Nicholas et al., 1998, "A Tutorial on Searching Sequence Databases and Sequence Scoring Methods" ~~(www.psc.edu)~~ and references cited therein.; W.R. Pearson, 1991, Genomics 11:635-650). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff (Dayhoff: Atlas of Protein Sequences and Structure, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA), and normalized by Gribskov (Gribskov 1986 Nucl. Acids Res. 14(6):6745-6763). The Smith-Waterman algorithm may be employed where default parameters are used for scoring (for example, gap open penalty of 12, gap extension penalty of two). From the data generated, the "Match" value reflects "sequence identity."

Please replace the fourth paragraph on page seven of the specification with the following substitute paragraph:

In other embodiments, moderately stringent hybridization conditions are used that comprise: pretreatment of filters containing nucleic acid for 6 h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HC1 (pH7.5), 5mM EDTA, 0.1% PVP, 0.1% Ficoll®, 1% BSA, and 500 µg/ml denatured salmon sperm DNA; hybridization for 18-20h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCI (pH7.5), 5mM EDTA, 0.02% PVP, 0.02% Ficoll®, 0.2% BSA, 1001..tg/m1 salmon sperm DNA, and 10% (wt/vol) dextran sulfate; followed by washing twice for 1 hour at 55° C in a solution containing 2X SSC and 0.1% SDS.

Please replace the paragraph [0071] of the specification with the following substitute paragraph:

Hypoxic induction. The alpha subunit of the transcription factor, hypoxia inducible factor-1 (HIF-1), is upregulated in tumor cells following exposure to hypoxia in vitro. Under hypoxic conditions, HIF-1 stimulates the expression of genes known to be important in tumour cell survival, such as those encoding glyolytic enzymes and VEGF. Induction of such genes by hypoxic conditions may be assayed by growing cells transfected with PAK in hypoxic conditions (such as with 0.1% 02, 5% CO2, and balance N2, generated in a Napco® 7001 incubator (Precision Scientific)) and normoxic conditions, followed by assessment of gene activity or expression by Taqman®. For example, a hypoxic induction assay system may comprise a cell that expresses a PAK, and that optionally has defective CHK function (e.g. CHK is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the hypoxic induction assay system and changes in hypoxic response relative to controls where no test agent is added, identify candidate CHK modulating agents. In some embodiments of the invention, the hypoxic induction assay may be used as a secondary assay to test a candidate CHK modulating ~~agents~~ agent that is initially identified using another assay system. A hypoxic induction assay may also be used to test whether PAK function plays a direct role in the hypoxic response. For example, a hypoxic induction assay may be performed on cells that over- or under-express PAK relative to wild type cells. Differences in hypoxic response compared to wild type cells suggests that the PAK plays a direct role in hypoxic induction.

Please replace the fourth paragraph on page 29 of the specification with the following substitute paragraph:

For nucleic acid modulators, primary assays may test the ability of the nucleic acid modulator to inhibit or enhance PAK gene expression, preferably mRNA expression. In general, expression analysis comprises comparing PAK expression in like populations of cells (e.g., two pools of cells that endogenously or recombinantly express PAK) in the presence and absence of the nucleic acid modulator. Methods for analyzing mRNA and protein expression are well known in the art. For

instance, Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR (e.g.,. using the TaqMan®, PE Applied Biosystems®), or microarray analysis may be used to confirm that PAK mRNA expression is reduced in cells treated with the nucleic acid modulator (e.g., Current Protocols in Molecular Biology (1994) Ausubel FM *et al., eds.,* John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.,* Biotechniques (1999) 26:112125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm DH and Guiseppi-Elie, A. Curr Opin Biotechnol 2001, 12:41-47). Protein expression may also be monitored. Proteins are most commonly detected with specific antibodies or antisera directed against either the PAK protein or specific peptides. A variety of means including Western blotting, ELISA, or in situ detection, are available (Harlow E and Lane D, 1988 and 1999, *supra).*

Please replace the first paragraph on page 31 of the specification with the following substitute paragraph:

In a preferred embodiment, CHK pathway activity is assessed by monitoring neovascularization and angiogenesis. Animal models with defective and normal CHK are used to test the candidate modulator's affect on PAK in ~~Matrigel~~ MATRIGEL® assays. ~~Matrigel~~ MATRIGEL® is an extract of basement membrane proteins, and is composed primarily of laminin, collagen IV, and heparin sulfate proteoglycan. It is provided as a sterile liquid at 4° C, but rapidly forms a solid gel at 37°C. Liquid ~~Matrigel~~ MATRIGEL® is mixed with various angiogenic agents, such as bFGF and VEGF, or with human tumor cells which over-express the PAK. The mixture is then injected subcutaneously (SC) into female athymic nude mice (Taconic, Germantown, NY) to support an intense vascular response. Mice with ~~Matrigel~~ MATRIGEL® pellets may be dosed via oral (PO), intraperitoneal (IP), or intravenous (IV) routes with the candidate modulator. Mice are euthanized 5 – 12 days post-injection, and the ~~Matrigel~~ MATRIGEL® pellet is harvested for hemoglobin analysis (Sigma plasma hemoglobin kit). Hemoglobin content of the gel is found to correlate with the degree of neovascularization in the gel

Please replace the second paragraph on page 37 of the specification with the following substitute paragraph:

All cell lines used in the following experiments are NCI (National Cancer Institute) lines, and are available from ATCC℠ (American Type Culture Collection, Manassas, VA 20110-2209).

Normal and tumor tissues were obtained from Impath, UC Davis, Clontech™, Stratagene®, and Ambion®.

Please replace the third paragraph on page 37 of the specification with the following substitute paragraph:

TaqMan® analysis was used to assess expression levels of the disclosed genes in various samples.

Please replace the fourth paragraph on page 37 of the specification with the following substitute paragraph:

RNA was extracted from each tissue sample using Qiagen™ (Valencia, CA) RNeasy® kits, following manufacturer's protocols, to a final concentration of 50ng/g1. Single stranded cDNA was then synthesized by reverse transcribing the RNA samples using random hexamers and 500ng of total RNA per reaction, following protocol 4304965 of Applied Biosystems® (Foster City, CA).

Please replace the fifth paragraph on page 37 of the specification with the following substitute paragraph:

Primers for expression analysis using TaqMan® assay (Applied Biosystems®, Foster City, CA) were prepared according to the TaqMan® protocols, and the following criteria:

a) primer pairs were designed to span introns to eliminate genomic contamination, and

b) each primer pair produced only one product. Expression analysis was performed using 7900HT instrument.

Please replace the sixth paragraph on page 37 of the specification with the following substitute paragraph:

TaqMan® reactions were carried out following manufacturer's protocols, in 25 R1 total volume for 96-well plates and 10 total volume for 384-well plates, using 300nM primer and 250 nM probe, and approximately 25ng of cDNA. The standard curve for result analysis was prepared

using a universal pool of human cDNA samples, which is a mixture of cDNAs from a wide variety of tissues so that the chance that a target will be present in appreciable amounts is good. The raw data were normalized using 18S rRNA (universally expressed in all tissues and cells).